International Journal of Drug Delivery 3 (2011) 642-647

http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Preparation and Evaluation of Diclofenac Potassium loaded Agarose Beads.

Anindya Kishore Maiti[1]*, B Mazumdar[2], A K Dhara[1], A Nanda[3]

*Corresponding author:

Anindya Kishore Maiti

[1]Department of Pharmacy, Contai Polytechnic, Contai-721 401 India

[2]Department of Pharmaceutical Sciences, Dibrugarh University, Assam. India

[3]Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032 India

Abstract

Controlled release technology has emerged to be an important area of research in the field of novel drug delivery system. Being a member of controlled release system, microspheres are very useful for achieving therapeutic response for improving patient compliance. Microspheres of diclofenac potassium were prepared by using cold gelation technique with the help of a biodegradable polymer-agarose. Beads were prepared by dropping a hot aqueous agar solution in to a beaker containing a mixture of chilled light liquid paraffin and water. pH of water was adjusted 1.60 by adding concentrated hydrochloric acid to reduce the prior diffusion of diclofenac potassium. Drug release profile (*in-vitro* release), swelling index, particle size analysis, entrapment of drug into beads were investigated. Scanning electron microscopy (SEM) was carried out for characterisation of beads.

Keywords: Microsphere; Diclofenac potassium; Agarose; SEM.

Introduction

Controlled release technology now forms the essence of modern and future drug delivery system for last several decades in terms of clinical efficacy and patient compliances[1]. For this purpose, agar, a natural polymer is used for preparation of diclofenac potassium microsphere by a modified gelation method[2]. Natural polymers have lots of advantages over synthetic polymers as safe and non-toxic in nature, highly bio-compatible and bio-degradable, very much environment and eco-friendly. Some organic materials are required to dissolve synthetic polymers, which causes possible toxicity, explosion hazards[3]. Agar is prepared from various species of *Geledium amansii* and other red algae. It has molecular weight 120000, gelling point 35^0C to 45^0C for 1.5%(w/v). At 90^0C agar forms a colloidal substance with water and forms a solid gel upon cooling below its gelling point. This property tends a suitable balance between easy melting and good gel stability at relatively high temperatures. In the gel agar forms double helices which linked together forms bundle of network[4].

Diclofenac potassium (DP) is a mono potassium salt of benzoic acetic acid derivative (mol. wt 334.25). It is a very popular and most useful non-steroidal anti-inflammatory agent which is widely use in rheumatoid arthritis[5] and other inflammatory conditions[6]. It is more potent than diclofenac sodium[7]. The biological half life of diclofenac potassium is about 1-2 hours and the usual oral dose is 50mg, 2-4 times a day[8]. Therefore it requires multiple dosing to maintain therapeutic blood level of the drug. The most frequent adverse side effects of diclofenac potassium on long term administration are gastrointestinal disturbances, hyper acidity, peptic ulceration and gastrointestinal bleeding[9-10]. For this purpose agarose beads were prepared for control drug delivery system, increasing surface area and incorporating cross linking divalent metal ($CaCl_2$) into agar beads.

Materials and Methods

Materials

Diclofenac potassium IP was received as a gift sample from U S Vitamins Ltd, Gujarat, India. Agarose and calcium chloride (analytical grade) were purchased from Loba Chemicals and Qualigens India Ltd, respectively. All other ingredient used of analytical grade.

Methods

Preparation of microbeads

Agar coated diclofenac potassium beads were prepared by modified technique followed by work of E.A.El.Fattah[11]. At first a

gelation medium was prepared by dissolving different quantities of agar (W/V) with water and heated slowly. Drug polymer solution was prepared by dispersing the drug slowly into previously prepared agar gel with continuous and uniform stirring for 3hours. Bubble free dispersion medium was extruded through glass syringe (20G) in a drop wise fashion into the beaker which is filled with chilled acidified water (pH 1.6), $CaCl_2$ and liquid paraffin. The agitation was carried out by magnetic stirrer at 200rpm.when hot gel was poured in to chilled paraffin, the droplet become sphere due to paraffin and hard due to the temperature below gelling point of agar. After completion of beads formation the beads were removed by decanting the paraffin and acidified water mixture and washed with hexane to remove the paraffin. The beads were dried at 30^0C under reduced pressure till they attained constant weight. This method was repeated for different formulations by changing the quantities of agar and $CaCl_2$.

Yield of beads

To determine the yield following formula was employed.

Yield of beads= —— X 100

Where W_1 = the weight summation of drug, polymers and cross linking agents, W_2 = the weight of the beads prepared experimentally. The mean of three determinations was reported in Table-2.

Determination of drug entrapment efficiency

To evaluate the drug content inside the beads, the digestion method was applied. The drug – loaded beads (equivalent to 50mg) were pulverized and incubated in 50ml of 0.1N NaOH solution at room temperature for 24h. After the interval the solution was stirred, filtered, diluted and assayed spectrophotometically at 276nm.

The drug entrapment efficiency = —— X 100

Where X_1 = the theoretical amount of the drug present in the beads, X_2 = the experimental amount of the drug present in the beads. The mean of three determinations was reported in Table-2.

Particle size analysis

The particle sizes of the prepared microbeads were determined using the optical microscopy method. It was the most direct method for size distribution measurement. The prepared microbeads were mounted in light liquid paraffin and the diameters of 100 particles were measured by means of an optical microscope equipped with a calibrated ocular micrometer. Then the mean diameter was calculated and represented in Table-2

In vitro release study

The dissolution profile of DP- microsphere was determined by using USP (Type-I) basket type dissolution test apparatus taking 900ml of phosphate buffer (pH 7.4) solution. The study was carried out for eight hours. The dissolution medium was maintained at a temperature of 37 ± 1^0C. The basket was covered by 100 mesh nylon cloth and rotated at 50 rpm. The 10ml of sample was taken at every 1h interval and simultaneously equal quantity of the corresponding blank dissolution medium was added to the dissolution apparatus. The sample was filtered and suitably diluted to determine the absorbance at 276nm in double beam UV spectrophotometer (CHEMITO-2500). Cumulative percentages of drug release from the microbeads were determined at different intervals and plotted in figures.

Results and Discussions

The microbeads were sufficiently hard and spherical in shape. The beads were characterized for their particle size by microscopic method, drug loading efficiency and scanning electron microscopy.

Scanning Electron Microscopy (SEM) Analysis:

The surface analysis of drug loaded beads was done by Scanning electron microscope (Model JEOL JSM-6360, Japan). From the scanning electron microscopy analysis (Fig 1-2) it was found that, microspheres prepared by cold gelation method were spherical, non-aggregated and porous. The surface of the blank microsphere were smooth where as drug loaded placebos were slightly rough than blank placebos. The study of drug loaded beads showed the presence of drug particles on the surface and that might be factor for initial release of drug by bursting effect.

Effect of polymer concentration on *in-vitro* DP release from agar beads:

The *in-vitro* release patterns of diclofenac potassium in different polymer concentrations were studied and were shown in fig-3; here agar was used in all twelve formulations at a ratio 1:1, 1:2 and 1:3. The release pattern showed the initial bursting effect, followed by slow release. Agar has a popular gelling effect, when it comes contact with buffer medium; it starts partially hydrated and forms a gel layer. More quantities of polymer provides increasing amount of viscous gel layers and soluble drugs were diffused from gel layer in a decreased rate fashion. It may happen as the drug release channel length may increase with reduced porosity and high tortuosity as gel layer was increased. This factor can be attributed to the reduction of release rate with increment of polymer level of agar beads.

Effect of electrolyte concentration on *in-vitro* DP release from agar beads:

Calcium chloride was used as electrolyte at different concentration in all twelve formulations. Formula code F1, F2, F3 contains 4%, Formula code F4, F5, F6 contains 6%, Formula code F7, F8, F9 contains 8% and Formula code F10, F11, F12 contains 10% of electrolytes. Release rate of drug increased with the increasing amount of the electrolyte followed by first group released 62% (max) and 50%(min) where as last group released 87%(max) and 77%(min)(Fig-4). It may happen due to salting out of electrolytes. When beads containing electrolyte come contact to buffer system, electrolyte dissolve out rapidly from beads creating channels through which dissolution medium can get into and leach the drug out of the beads.

Conclusion

Diclofenac potassium loaded microspheres were prepared successfully by cold gelation method using the combination of agar and calcium chloride in different ratios. It was observed that the prepared microspheres were spherical, free flowing, high percentage entrapment efficiency and high percentage of yielding capacity.

The *in-vitro* controlled release of DP from the prepared microspheres formulations have been established in this study. It also might be said that higher concentration of agarose along with lower percentage of electrolytes had a higher retarding capacity. However, the *in-vitro* release characteristics of the drug from the microspheres are subject to confirmation in animal and human studies for coming into conclusion of enhanced bioavailability and reduced dose frequency to improve patient compliance, which is under progress in our laboratory.

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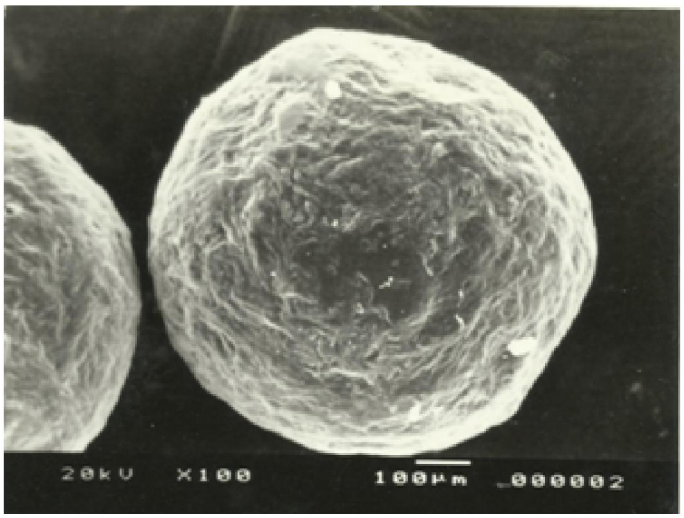

Figure 1: SEM photograph (X100) of Diclofenac Potassium loaded agar bead.

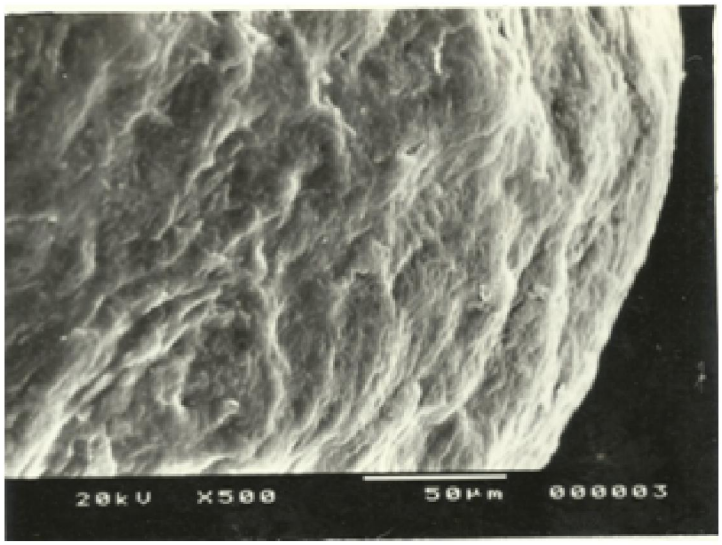

Figure 2: SEM photograph (x500) of DP Loaded agar bead before dissolution.

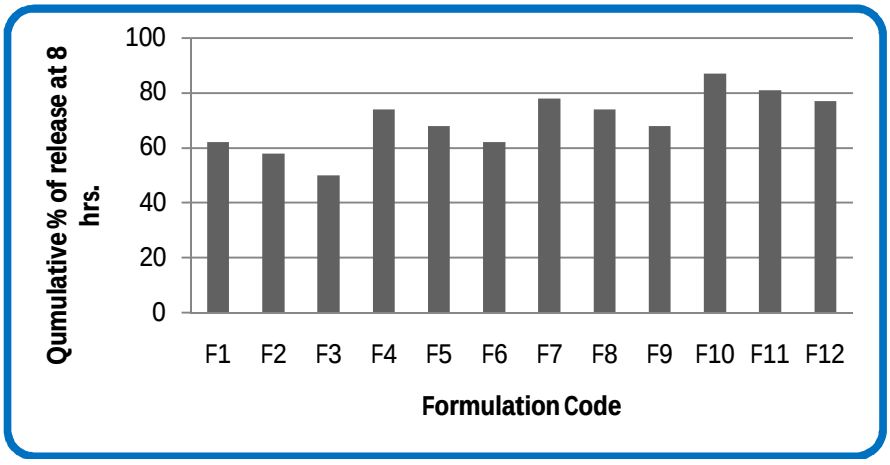

Figure 3: Effect of polymer on release rate of twelve formulations of agarose beads.

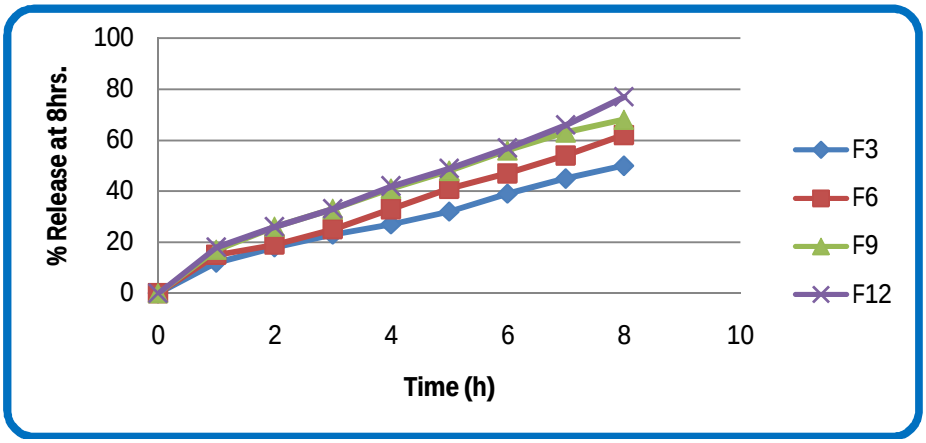

Figure 4: Effect of electrolyte on release rate of four formulations of agarose beads.

Table 1 : Quantities of different ingredients used in preparing microbeads.

Formulation Code	Diclofenac potassium (mg)	Agar (mg)	Cross linking Agent ($CaCl_2$) (%)	Stirring speed (rpm)
F1	200	200	4	200
F2	200	400	4	200
F3	200	600	4	200
F4	200	200	6	200
F5	200	400	6	200
F6	200	600	6	200
F7	200	200	8	200
F8	200	400	8	200
F9	200	600	8	200
F10	200	200	10	200
F11	200	400	10	200
F12	200	600	10	200

Table 2: Percentages of yield and the drug entrapment as well as the mean diameter of microbeads.

Bead Code	Yield (%)	Entrapment (%)	Mean Diameter (mm)
F1	79.25	92.30	0.78
F2	77.10	89.73	0.92
F3	72.44	88.33	0.93
F4	74.21	90.88	0.96
F5	86.63	88.32	0.98
F6	70.68	88.70	0.80
F7	68.36	83.26	0.85
F8	78.97	81.42	0.92
F9	72.38	89.47	0.96
F10	79.45	86.58	0.87
F11	83.12	84.25	0.89
F12	89.52	81.12	0.94